

Amendments to the Claims:

The following listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Amended) A method for the amplification of RNA, in a sample, comprising:
 - a) obtaining a starting solution by adding to a container comprising the sample, a buffer, a first primer, a second primer, a plurality of nucleotide triphosphates, a sufficient amount of an enzyme system having reverse transcriptase activity and a heat stable enzyme system having DNA polymerase activity, and closing the container after step a) and maintaining the container closed until the obtainment of the desired amount of amplified product, wherein said sufficient amount is an amount which, after the heat treatment of step b) below, will retain sufficient reverse transcriptase activity to permit performance of step c) hereafter;
 - b) heating the solution obtained in a) to a temperature sufficient to permit denaturation, said temperature not to exceed 75° C., and maintaining said temperature for a sufficient time to provide denaturation of said RNA without inactivating the enzymic system having reverse transcriptase activity;
 - c) bringing the solution obtained in b) to a predetermined temperature and maintaining said temperature for sufficient time whereby a first cDNA strand is synthesized and a RNA-cDNA heteroduplex is formed;
 - d) heating the solution obtained in c) to a predetermined temperature whereby said RNA-cDNA heteroduplex is denatured to form an RNA single strand and a first cDNA single strand;
 - e) bringing the solution obtained in d) to a predetermined temperature and maintaining said temperature for a sufficient time whereby the second primer hybridizes with the first cDNA strand;

f) bringing the solution obtained in e) to a predetermined temperature and maintaining said temperature for a sufficient time whereby a second cDNA strand is synthesized to form a double-stranded cDNA; and

g) denaturing the double-stranded cDNA and subjecting the cDNA strands to a sufficient number of amplification cycles to obtain a desired amount of amplified product;

wherein after step a), all steps are performed in the closed container, without subsequent addition of any ingredients.

22. (Amended) A method for the amplification of RNA in a sample in a closed container, comprising:

a) obtaining a starting solution by placing, in a container, the sample, a buffer, a first primer, a second primer, a plurality of nucleoside triphosphates, and an enzyme system having reverse transcriptase activity and DNA polymerase activity, and closing the container after step a) and maintaining the container closed until the obtainment of the desired amount of amplified product;

b) heat treating said solution at a temperature sufficient to permit denaturation of secondary structures that may be present in said RNA but not above 75° C., for a time sufficient to permit denaturation of secondary structures without completely inactivating the reverse transcriptase and DNA polymerase activities of said enzyme system;

c) permitting a first cDNA strand to be synthesized and a RNA-cDNA heteroduplex to be formed;

d) heat treating the solution containing said RNA-cDNA heteroduplex at a temperature at which said heteroduplex is denatured to form an RNA single strand and a first cDNA single strand without completely inactivating the DNA polymerase activity of said enzyme system;

e) permitting the second primer to hybridize with the first cDNA strand, followed by synthesis of a second cDNA strand to form a double-stranded cDNA; and

f) denaturing the double-stranded cDNA and subjecting the cDNA strands to a sufficient number of amplification cycles to obtain a desired amount of amplified product;
wherein after step a), all steps are performed in the closed container, without
subsequent addition of any ingredients.